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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
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| 10/622,240 | 07/18/2003 | George Tzertzinis | NEB-208/9-US | 3580 |

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| EXAMINER |
|---|
| POPA, ILEANA |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1633 | |

| SHORTENED STATUTORY PERIOD OF RESPONSE | MAIL DATE | DELIVERY MODE |
|---|---|---|
| 3 MONTHS | 01/04/2007 | PAPER |

**Please find below and/or attached an Office communication concerning this application or proceeding.**

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/622,240 | TZERTZINIS ET AL. | |
| | Examiner | Art Unit | |
| | Ileana Popa | 1633 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *10 October 2006*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☐ Claim(s) *1, 2, 5-7, and 12-47* is/are pending in the application.

4a) Of the above claim(s) *8,10,15,19 and 21-46* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2,5-7,9,12-14,16-18,20 and 47* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

## DETAILED ACTION

1. The text of those sections of Title 35, U.S. Code not included in this action can be found in the prior Office Action.

2. Claims 3, 4, and 11 have been cancelled. Claims 8, 10, 15, and 19 have been withdrawn as being drawn to non-elected species. Applicant withdrew claims 21-46. Claims 1, 2, 12, 13, and 47 have been amended. No new matter was introduced by these amendments.

Claims 1, 2, 5-7, 9, 12-14, 16-18, 20, and 47 are under examination.

### *Response to Arguments*

### *Oath/Declaration*

3. The objection to the oath/declaration is withdrawn in response to Applicant's submission of a new declaration identifying the instant application by its number and filing date on 10/10/2006.

### *Drawings*

4. The objection to the drawings for not being of sufficient quality is withdrawn in response to Applicant's submission of replacement drawings on 10/10/2006.

***35 USC § 102***

5. The rejection of claims 1-4, 11, 13, 14, 16-18, and 47 under 35 USC § 102(b) as being anticipated by Zamore et al. (Cell, 2000, 101: 25-33) is withdrawn in response to Applicant's amendments to the claims to recite a prokaryotic RNase III and cancellation of claims 3, 4, and 11 on 10/10/2006.

6. The rejection of claims 1, 2, 4-7, 9, 12, and 47 under 35 USC § 102(e) as being anticipated by Beach et al. (PGPUB 2002/0162126), as evidenced by Ketting et al. (Genes & Development, 2001, 15: 2654-2659) is withdrawn in response to Applicant's amendments to the claims to recite a prokaryotic RNase III and cancellation of claims 3 and 4 on 10/10/2006.

7. The rejection of claims 3 and 11 under 35 USC § 102(e) as being anticipated by Yang et al. (PGPUB 2004/0014113) is withdrawn in response to cancellation of claims 3 and 11 on 10/10/2006.

8. The rejection of claims 1, 2, 19, 12-14, 16-18, 20, and 47 under 35 USC § 102(e) as being anticipated by Yang et al. is withdrawn because Applicant amended the claim to recite a ratio of enzyme to substrate (w/w) greater or equal to 0.25:1 that is not taught by Yang et al. The amendment was filed on 10/10/2006. However, the amendment to the claims was not sufficient to overcome the rejection of the claims under 35 U.S.C. § 103(a) (see below).

***35 USC § 103(a)***

9. Claims 1, 2, 5-7, 9, 12-14, 16-18, 20, and 47 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Yang et al. in view of Gross et al. (Nucleic Acids Research, 1987, 15: 431-442) for the reasons of record set forth in the prior Office action. Applicants' arguments filed on 10/10/2006 have been fully considered but they are not persuasive.

Applicant traversed the instant rejection on the grounds that there is no support in Yang et al. for the limitation of the complete digestion being achieved in less than 6 hours. Applicant argues that Yang et al. teach that exhaustive cleavage of dsRNA by *E. coli* RNase III leads to short (i.e., 12-15 bp long) dsRNAs unable to trigger an RNAi response (p.2, paragraph 0015). Applicants asserts that a digestion of 6, 4, 2 hours, or even a few minutes in the presence of magnesium ions could significantly degrade dsRNA to a size unsuitable for RNAi and therefore there is no basis form the Examiner's assumption that Yang et al. reference describes or enables the production of hsiRNA. Applicant argues that, since Yang et al. teach their enzyme as highly active at 37C, it can be deduced that the method of Yang et al. would produce fragments that are too short to accomplish RNAi and they cite paragraph 0053 of Yang et al. to support this statement:

> "After optimization we found that limited RNase III digestion of dsRNA at room temperature for 1 hour yielded ample amounts of esiRNA for inhibition of most genes."

Applicant asserts that it is possible that, at room temperature, most material was undigested or degraded and it is likely that large amounts of starting material were

required to produce "ample amounts of esiRNA" and that RNAi appears to work sometimes since the term "most genes" is not defined. Applicants submits that the figures provided by Yang et al. do not help interpreting the above because agarose gels are poor discriminators of sizes for fragments less than 200 bp, and Fig. 1B (which presents a 4% agarose gel) shows gradients of bands of different sizes at 15 minutes incubation. Applicant submits that these results reflect the difficulty to provide enough RNase III to achieve any cleavage and the difficulty to prevent total cleavage of dsRNA into 12-15 bp fragments. Applicant also argues that "efficiently generating 20-25 bp siRNA" as taught by Yang et al. (p. 2, paragraph 0015) cannot be interpreted as meaning more than 30% because Yang et al. teach that exhaustive digestion results in ineffective products and that a limited digestion results in a smear that proves that the efficiency is at best 0.1 or 1% (Fig. 1 of Yang et al.). With respect to the limitations recited in claim 14, Applicant argues that Yang et al. make no reference to the portion of sequence represented by RNase III cleavage products. With respect to the combination of Yang et al. with Gross et al., Applicant submits that the Examiner's rejection is based on hindsight and in fact there is no motivation to support the combination because the references teach away from each other. Applicant argues that there is no suggestion in Yang et al. that a divalent transition metal ion can be used instead of the standard magnesium, that Gross et al. teach generating smaller fragments that otherwise obtained in the presence of magnesium ions, which is in contrast to Yang et al. who teach to avoid generating small fragments by performing limited digestions with RNase III in the presence of magnesium. Applicants conclude that, while Yang et al. seek to

reduce RNase III cleavage, Gross et al. seek to increase RNase III cleavage, and therefore the rejection should be withdrawn.

Contrary to Applicant's assertion, Yang et al. do teach that cleavage 15-30 bp fragments are visible within 1 minute and that by 3 minutes these fragments become the main products (i.e., complete digestion) when incubation is performed at 37C (p. 6, paragraph 0053, Fig. 1b, line 6). Therefore, Applicant's assumption that a 37 C digestion of a few minutes significantly degrades the dsRNA to a size unsuitable for RNAi is incorrect. Yang et al. also teach that efficient digestion can be performed at room temperature for 1 hour resulting in enough esiRNA for inhibition of a wide variety of genes such as abundant long-lived or rare short-lived transcripts/proteins and that esiRNA is a valuable tool for selective depletion of genes in mammalian cells (p. 6, paragraph 0053, p. 7, paragraph 0069, p. 8, paragraphs 0070 and 0071). Therefore, Applicant's assumption that RNAi appears to work only sometimes is incorrect, since Yang et al. clearly teach their method as being generally applicable for silencing in mammalian cells. Regarding the argument that Fig. 1B shows gradients of bands of different sizes, it is noted that these gradients represent the dsRNA to be cleaved and not the final product, i.e., esiRNA. Applicant is invited to take a more careful look at the figures in question. Fig. 1B represents an agarose gel analysis of the time-course of dsRNA digestion by RNase III; lanes 1, 2, 4, 5, 7, and 8 (i.e., the gradients of bands with different sizes or smears) are dsRNA to be digested (it is known in the art that RNA migrates as a smear on agarose gels), while lanes 3, 6, and 9 are esiRNA. It is clear from the figure that esiRNA migrates as a well-defined band and therefore, a limited

digestion does not result in a smear. Fig. 1C represents a polyacylamide gel analysis of esiRNA obtained after dsRNA digestion by RNase III, wherein lane M is 10 bp DNA marker, lanes 1 and 2 are controls (i.e., chemically synthesized siRNAs), lane 3 is 21-23 bp, lane 4 is 24-26 bp, and lane 5 is 27-30 bp esiRNA. It is clear from the figure that effective digestion was achieved and that the method does result in 21-30 bp esiRNA and not in unsuitable 2-15 bp fragments, as asserted by the Applicant (seep. 1, paragraph 0005, p. 6, paragraph 0053,and Fig. 1B and C). With respect to the limitations of the siRNAs pool representing a substantial portion of the sequence of a large dsRNA from which it is derived, since the digestion is complete (see above), the esiRNA of Yang et al. meets this limitation.

In response to applicant's argument that the Examiner's conclusion of obviousness is based upon improper hindsight reasoning, it must be recognized that any judgment on obviousness is in a sense necessarily a reconstruction based upon hindsight reasoning. But so long as it takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only from the applicant's disclosure, such a reconstruction is proper. See *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971). With respect to the argument that Yang et al. and Gross et al. teach away from each other, it is noted that Gross et al. teach more efficient cleavage and not generation of smaller, unsuitable fragments. Gross et al. teach that lower monovalent salt concentration results in the cleavage of additional secondary sites by *E. coli* RNase III and that more secondary sites are cleaved if magnesium is replaced with manganese

(p. 431, p. 432, first paragraph, p. 4339, first paragraph, Fig. 4b). Therefore, Gross et al. teach that cleavage by *E. coli* RNase III can be rendered more efficient by changing both monovalent ion concentration and exchanging magnesium with manganese. It is not clear why Applicant asserts that Yang et al. use higher salt concentrations to limit the cleavage, because Yang et al. do not teach such a thing. However, since Gross et al. teach that cleavage at low monovalent concentration and in the presence of manganese is specific rather than random (i.e., the secondary sites are identical to the primary sites recognized by the *E. coli* RNase III in conventional conditions) (p. 432, first paragraph, p. 441), one of skill in the art would have known that using the digestion conditions taught by Gross et al. would not result in unsuitable fragments for RNAi. Moreover, one of skill in the art would have been motivated to change the method of Yang et al.by using the digestion conditions of Gross et al. because by doing so, one of skill in the art would have expected a more efficient digestion. With respect to the limitation of a ratio of enzyme to substrate (w/w) greater or equal to 0.25:1, this is not innovative over the prior art. The art teaches the utility of using a range of different ratios for the identification of optimal reaction conditions and that this can be accomplished by routine experimentation.

Thus, the claimed invention was *prima facie* obvious at the time the invention was made.

## *Conclusion*

10. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Ileana Popa whose telephone number is 571-272-5546. The examiner can normally be reached on 9:00 am-5:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach can be reached on 571-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300..

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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